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Screening of *Wrightia tinctoria* leaves for Anti psoriatic activity

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Abstract

***Plan**: The hydro alcoholic extract of Wrightia tinctoria leaves was evaluated for antipsoriatic activity by mouse tail test.*
***Methodology**: Antipsoriatic activity was performed at a dose 200 mg/kg body weight in mice (25-30 g). Isoretinoic acid (0.5 mg/kg) was used as the standard. Degree of orthokeratosis, drug activity and the relative epidermal thicknesses were calculated and statistically analyzed. The extract was also evaluated for its antioxidant potential by DPPH, nitric oxide and hydrogen peroxide radical scavenging assays.*
***Outcome**: The extract produced significant ($p<0.01$) degree of orthokeratosis compared to control and the drug activity was found to be 70.18%, which is more potent than the standard (57.43%).. The extract showed prominent antioxidant activity in all the assays. The present study concludes that the selected plant has antipsoriatic activity and can be used for psoriasis treatment.*
***Key words**: Psoriasis, Wrightia tinctoria, Antioxidant, hydro alcoholic extract, Antipsoriatic activity.*

Introduction

Psoriasis is a chronic recurrent disease affecting the skin, nails, and joints. Between 0.6–4.8% of the general population is affected with psoriasis. It is grouped under the papulo-squamous disorders. Serious research is ongoing worldwide for effective management of the disease. Currently available therapeutic options include topical (emollients, moisturizers, tars, anthralins, topical corticosteroids, vitamin A analogs, and vitamin D analogs), systemic treatments (corticosteroids, methotrexate, cyclosporine, etretinate (retinoids) and other immunomodulators, and hydroxyurea), phototherapy and photo-chemotherapy[1]. Biologic drugs have recently been approved by the US Food and Drug Administration (FDA) [2], for the treatment of severe psoriasis. Affordability, availability, and side effects of extended use of the above therapies still remain a challenge and concern.

In general, herbal formulations are less expensive than the above therapies and are known to minimize the risk of side effects; they therefore provide a viable alternative for psoriasis management. Several herbal formulations are in clinical use in the Indian system of traditional medicine to treat skin diseases, but they are often undocumented and not clinically or scientifically validated. There are increasing research efforts to develop herbal formulations to treat psoriasis, and there is a continuing need to develop herbal formulations to treat psoriasis effectively with minimal or no side effects.

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Antipsoriatic activities of this in plant emulsion form and in combination with other herb were already reported[3,4]. These studies showed the topical efficacy of *Wrightia tinctoria.* In the present study we evaluated the leaves of *Wrightia tinctoria* for antipsoriatic activity by oral administration in the mouse tail test, which is not reported elsewhere.

2. Materials and Methods

2.1. *Plant material*

Wrightia tinctoria leaves were collected locally and authenticated at the Botanical Survey of India, Coimbatore, Tamilnadu. The specimen sample (JSS/Phyto&Med/230/12) was deposited at the herbarium of the Dept.of Phytomedicine and Phytopharmacy, JSS College of Pharmacy,Ooty, Nilgiris,Tamilnadu.

2.2. *Extraction*

Leaves of *Wrightia tinctoria* were dried and powdered. It was extracted with 50% ethanol by hot decoction method. The extract obtained concentrated under vacuo and dired in an oven at 40°C to yield the dry extract (17.35% w/w).

2.3. *Antioxidant studies*

2.3.1. *Diphenyl picryl hydrazyl (DPPH) radical scavenging assay*

Assay was carried out in a 96 well microtitre plate[5]. To 10 µl of test or standard solution 100 µl of DPPH solution was added in wells of the microtitre plate. The final concentration of the test and standard solutions used were from 1000 to 1.95 µg/ml. The plates were incubated at 37°C for 20 minutes and the absorbance of each well was measured at 490 nm, using ELISA reader against the corresponding blank solutions. Activity at the tested concentrations were calculated using the formula,

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

IC_{50} (Concentration of the sample required to scavenge 50% of DPPH radicals) value of each sample was calculated by plotting a graph between concentration and percentage inhibition.

2.3.2. *Nitric oxide radical inhibition assay*

The reaction mixture (6 ml) containing sodium nitroprusside (10 mm, 4 ml), phosphate buffered saline (1 ml) and 1 ml of sample in DMSO were incubated at 25°C for 150 minutes. After incubation 0.5 ml of the reaction mixture was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of NEDD was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature.

The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution[5].

2.3.3. *Scavenging of Hydrogen peroxide radicals*

Various concentrations of the extracts, the compound and standard in methanol (1 ml) were added to 2 ml of hydrogen peroxide solution. After 10 minutes the absorbance was measured at 230 nm against corresponding blank solutions[5].

2.4. *Animals*

Wistar rats (150 – 200 g) and albino mice (25-30 g) were used in this study. They were procured from the animal house JSS College of Pharmacy, Ooty and fed with standard diet and water *ad libitum*. All animal experiments were performed as per the CPCSEA guidelines and the experimental protocol was approved by the Institutional Animal Ethics Committee, JSS College of Pharmacy, Ooty.

2.5. *Acute toxicity study*

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines in mice at the dose level of extract up to 2000 mg/kg body weight. Acute toxicity studies of the extracts of plants were carried out as mentioned below[6].

The mice were fasted for overnight and maintained with water. The mice were separated into 3 groups of 6 animals each. The extract was administered at a dose level of 2000 mg/kg body weight orally as a fine suspension in 0.3% CMC in a volume of 2 ml/kg body weight. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hrs and 24 hrs to detect changes in autonomic or behavioral responses and also for tremor, convulsion, salivation, diarrhoea, lethargic sleep and coma. And then it was been monitored for any mortality for 14 days. A group of animals treated with vehicle (0.3% CMC) alone served as control.

2.6. *Anti psoriatic activity*

2.6.1. *Mouse tail test for psoriasis*

Wrightia tinctoria extract was evaluated for antipsoriatic activity by the mouse tail test for psoriasis. Eighteen animals (albino mice, 25-30 g) were divided into 3 groups of six each. Group I served as normal control (Water), group II served as positive control (isoretinoic acid, 0.5 mg/kg) and group III was treated with the extract at 200 mg/kg body weight. Samples were prepared in water. Treatment was performed once daily by oral administration for 14 days. At the end of the 14th day treatment, mice were sacrificed by deep ether anesthesia and the proximal parts of their tails were cut and each group tails stored in separate containers containing 10 % formalin in saline.

2.6.2. *Histopathological examination*

Longitudinal histological sections were prepared from the tail skin and stained with hematoxylin-eosin. The specimens were histometrically analyzed for: (1) the horizontal length of an individual scale lying in between adjacent hair follicles including sebaceous glands (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), (2) the horizontal length of the fully developed granular layer within an individual scale (n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 60 measurements per treatment), and (3) the vertical epidermal thickness between the dermo-epidermal junction and the lowest part of the stratum corneum (n = 5 measurements per scale, n = 10 scales per animal, n = 6 animals per treatment group; i.e. a total of 300 measurements per treatment)[7]. From these raw data (1 to 3) the following parameters were calculated according to Bosman *et al*.,[8] (4) the degree of orthokeratosis of an individual scale defined as the percentage ratio of (2) divided by (1) (n = 60 data per treatment condition), (5) the control related 'drug activity' upon epidermal differentiation,

$$\text{Drug activity} = \frac{OKs - OKc}{100 - OKc} \times 100$$

with OK (i.e. orthokeratosis) as the mean of the parameter explained under (4) for a test substance (s) and the untreated control condition (c), respectively, and (6) the relative epidermal thickness of individual scales as the percentage ratio of the measure under (3), for a given treatment in relation to the mean of untreated controls set to 100% (n = 300 data per treatment condition).

Taken together, from these calculations, the following three overall parameters were eventually used for the evaluation of the drug effects: (a) the degree of orthokeratosis, (b) the so-called 'drug activity' according to Bosman *et al*.,[8] and (c) the relative epidermal thickness. The data are presented as weighed mean values ± standard error. For statistical comparisons, explorative probabilities were obtained by the Mann-Whitney U test[7].

3. *Statistical analysis*

In the mouse tail test, for statistical comparisons, explorative probabilities were obtained by the Mann-Whitney U test. Results of all studies are given as weighed mean ± standard error.

4. Results and Discussion

The extract showed very good antioxidant activity in DPPH, H_2O_2 and nitric oxide scavenging assays with IC_{50} value of 14.12 ± 0.71 µgm/ml, 34.48 ± 5.84 µg/ml and 71.47 ± 5.95 µgm/ml respectively (Table 1). Ascorbic acid and rutin were used as standard in these assays to validate the results of the antioxidant potential of the extract. Antioxidant potential of a sample in important in any disease and is same with psoriasis, which is a chronic inflammatory skin disorder. The antioxidant potential of the extract may increase the antipsoriatic potential of the extract.

The mouse tail test first described by Jarrett and Spearman (1964), with certain modifications reported by other authors is a morphometry-based, relatively sensitive and well reproducible method. It allows the quantitative evaluation of the effects of antipsoriatic drugs on epidermal differentiation crucially disturbed in psoriasis[4,7,8]. This model is based on the induction of orthokeratosis in those parts of the adult mouse-tail, which have normally a parakeratotic differentiation.

Data for the degree of orthokeratosis and the relative epidermal thicknesses for the extract are presented in Table 2. Statistical analysis for the main parameter, i.e. degree of orthokeratosis, showed that the extract has significant ($p < 0.01$) efficacy (70.18 ±1.92%) in the induction of epidermal differentiation with respect to control.

Table 1. Results of i*n vitro* antioxidant studies

Samples	*IC_{50} values (µg/ml, Mean ± SD; n=3)*		
	DPPH	*Nitric oxide*	*H_2O_2*
Wrightia tinctoria extract	14.12 ± 0.71	71.47 ± 5.95	34.48 ± 5.84
Ascorbic acid	03.28 ± 0.21	-	-
Rutin	11.25 ± 0.29	68.44 ± 2.56	36.16 ± 0.16

Each value represents the mean ± standard error; **p < 0.01 vs control.

Table 2. Effect of extract on the degree of orthokeratosis and relative epidermal thickness as well as the 'drug activity' in the mouse tail test

Groups	*Degree of orthokeratosis (%)*	*Drug activity (%)*	*Relative epidermal thickness (%)*
Control	17.30 ± 4.09	-	100.00 ± 10.7
Standard	57.43 ± 5.13**	48.52	126.30 ± 03.8
Wrightia tinctoria extract	70.18 ±1.92**	63.94	138.50 ± 11.3

Each value represents the mean ± standard error; **p < 0.01 vs control.

These evaluations were re-emphasized when considering 'drug activity' as an overall parameter related to the control which is shown in the middle column of Table 2. The extract has shown potent activity (63.94%) than the standard isoretinoic acid (48.52%). Both the standard and sample increased the epidermal thickness compared to control in the mouse tail test. Representative histological section of the mouse tail skin per group is shown in Figure 1.

From the present study it can be concluded that oral administration of the hydroalcoholic extract of leaves of *Wrightia tinctoria* will be a useful remedy for psoriasis patients. However, the safety of the extract should be evaluated in chronic toxicity studies (oral) before it is taken for psoriasis treatment.

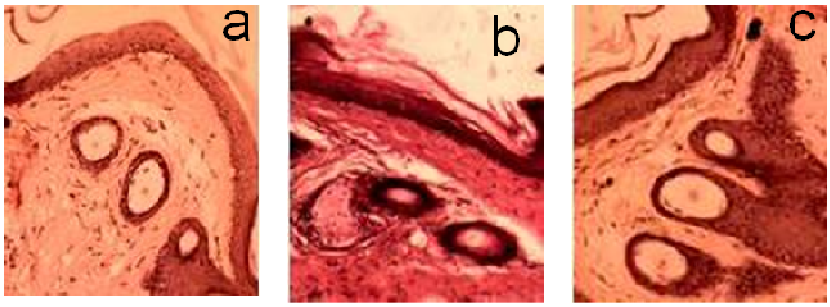

Figure 1. Longitudinal histological section of mouse tail skin at 40x, a) Control; b) Treated with Isoretinoic acid; c) Treated with *Wrightia tinctoria* extract

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References

1. Fox LP, Merk HF, Bickers DR. Dermatological pharmacology, in: Brunton LL, Laze JS, Parker KL, editors, Goodman and Gilman's The pharmacological basis of therapeutics. 11th ed., New Delhi: McGraw-Hill Medical Publishing Division; **2005**. p. 1679-1706.
2. Krueger G, Ellis CN, Psoriasis recent advances in understanding its pathogenesis and treatment, *J Am Acad Dermatol* ,**2005**, 94-100.
3. Baktha Reddy NB, Clinical and Histopathological Evaluation of the effect of *Wrightia tinctoria* formulation (RegSoR®) on Psoriasis Vulgaris, *US Dermatology Review,* **2007**, 6-8.
4. Mitra S.K., Seshadri S.J., Venkataranganna M.V. and Gopumadhvan S, Reversal of parakeratosis, a feature of psoriasis by *Wrightia tinctoria* (in emulsion) histological evaluation based on mouse tail, *Ind J Dermatol*, 43(3), **1998**, 102-104.
5. Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Antioxidant activity of *Caesalpinia digyna* root, '*J Ethnopharmacol*' 113, **2007**, 284-291.
6. Diener W, Mischke U, Kayser D, Schlede E, The biometric evaluation of the OECD modified version of Acute-Toxic-Class method (Oral), '*Arch Toxicol'*, 69, **1995**, 729-734.
7. Vogel GH. Drug Discovery and Evaluation. Second Edition, Springer, Germany, **1996**, 759-761.
8. Bosman B, Matshiesen T, Hess V, Frideriche E, A quantitative method for measuring antipsoriatic activity of drugs by the mouse tail test, '*Skin Pharmacol'*, 5, **1992**, 41-48.